Microtubule associated protein 1 light chain 3B (LC3/ATG8) functions in autophagosome formation and autophagy substrate recruitment. LC3 exists in both a soluble (autophagosome-independent) form as well as a lipid modified form that becomes tightly incorporated into autophagosomal membranes. Although LC3 is known to associate with tens of proteins, relatively little is known about soluble LC3 aside from its interactions with the LC3 lipid conjugation machinery. In previous studies we found autophagosome-independent GFP-LC3 diffuses unusually slowly for a protein of its size, suggesting it may be constitutively associated with a high molecular weight complex, form homo-oligomers or aggregates, or reversibly bind microtubules or membranes. To distinguish between these possibilities, we characterized the size, stoichiometry, and organization of autophagosome-independent LC3 in living cells and in cytoplasmic extracts using Fluorescence Recovery After Photobleaching (FRAP) and Fluorescence Polarization Fluctuation Analysis (FPFA). We found that the diffusion of autophagosome-independent LC3 was unaffected by either mutational disruption of its lipid modification or microtubule depolymerization, suggesting this form of LC3 does not reversibly bind to microtubules or membranes. Brightness and homoFRET analysis indicate LC3 does not homo-oligomerize, ruling out this as a possibility for its slow diffusion. In contrast, mutation of specific residues on LC3 required for binding other proteins and mRNA led to changes in the effective hydrodynamic radius of the protein as well as its stoichiometry. This suggests that LC3 associates with a multi-component complex consisting of either proteins or RNA. We conclude that autophagosome-independent LC3 associates with a complex with an effective size of ~500 kDa in the cytoplasm. These findings provide new insights into the nature of autophagosome-independent LC3 and illustrate the power of FRAP and FPFA to provide novel insights into the emergent properties of protein complexes in the autophagy pathway.

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Homeostasis of the Cellular Actin Cortex

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The cell cortex is a thin network of actin, myosin motors, and associated proteins that underlies the plasma membrane in most eukaryotic cells. It enables cells to resist extracellular stresses, perform mechanical work, and change shape. The actin network undergoes constant reorganisation due to molecular turnover. Hence, cortical structural and mechanical properties depend strongly on the relative turnover rates of its constituents and the actin filament length-distribution, but quantitative data on these dynamics remains elusive. We combined single molecule speckle microscopy and photobleaching experiments with microscopic computer simulations to analyse how molecular binding dynamics of G-actin to filaments sets network turnover and consequently the mechanical properties of the cellular actin cortex in living cells. Using photobleaching experiments, we found that two filament families with very different turnover rates composed the actin cortex: one with fast turnover dynamics and polymerisation resulting from addition of monomers to free barbed-ends and one with slow turnover dynamics with polymerisation resulting from formin-mediated filament growth. We show that filaments in the second subpopulation are on average longer than those in the first and that cofilin-mediated severing of formin-capped filaments contributes to replenishing the filament subpopulation with free barbedends. Additionally, we measured the molecular association rates and the distribution of travel-distances of actin monomers and formin dimers in speckle experiments and showed that this travel-distance distribution is consistent with the actin filament length-distribution found from photobleaching experiments and molecular simulations. Together, our results provide a quantitative characterisation of essential mechanisms underlying actin cortex homeostasis.

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Structural Transitions of Membrane-Bound Chiral Biopolymers

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In vivo, structural biopolymers such as MreB, FtsZ and eukaryotic homologs such as F-actin can exist in a membrane bound state where they assist in and regulate many important cellular functions including cell division and cell wall growth and maintenance. Here we show that the interplay between the chirality of a filament, its elasticity and membrane interactions can have non-trivial consequences for the conformations that it can adopt, thus directly affecting its functional role. We study a continuum model that describes a filament that is bound to an attractive cylindrical (rigid) membrane which extends our analysis for filaments bound to flat substrates (ref-2012). In the model we explicitly treat the natural preferred twist that is inherent in most biopolymers due to monomer stacking and assume that binding domains along the filament follow this natural twist forming a "sticky" attractive helix. We also introduce two elastic parameters allowing for the mechanical compliance of the filament, where filament bending along the surface of cylinder is modeled as a Worm-Like Chain (WLC) that couples to the curvature of the cylinder, and elastic twist deformation along the axis of the filament. We find, for certain parameter regimes, there exists a transition from an absorbed straight filament to a mechanically stable conformation where a helix is preferred. We also find there are periodic surface bound discommensurate stable solutions for both of the bending and twist degrees of freedom, where filament conformations are described by the well studied Frenkel-Kontorova dynamics for absorbed atomic lattices. Our results provide insight into the relation between a filament's molecular properties and its macroscopic conformation in a functional context.

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***In Vivo* Orientation of Single Myosins in a Zebrafish Embryo**

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Cardiac and skeletal myosin is highly organized in the muscle lattice where it powers contraction by transducing ATP free energy into the mechanical work of moving actin in a mechanism known as transduction/mechanical coupling. While muscle myosin can move actin *in vitro*, its *in vivo* environment is crowded and constrained by the fiber lattice. *In vivo*, myosin side chains are modified during- and post-translation by mutation, phosphorylation, deamidation, and oxidation under normal, diseased, or aging conditions and all potentially impacting transduction/mechanical coupling. Single myosin detection provides highly prized "bottom-up" quantitative characterization of myosin that tests hypotheses without the ambiguities inherent in ensemble derived observations. The marriage of *in vivo* and single myosin detection to study human cardiac or skeletal muscle contraction in zebrafish embryo models is a multi-scaled technology for basic and translational research. It allows one-to-one registration of a selected myosin molecular alteration with muscle filament-sarcomere-cell-fiber-tissue-organ- and organism levels of phenotype with confidence that all interactions and modifications are appropriately contributing their impact to myosin conformation. *In vivo* single myosin lever-arm orientation was observed at super-resolution using a photoactivatable GFP (PAGFP) tagged myosin light chain expressed in zebrafish skeletal muscle. Imaging was aided by an innovative microfluidic design for embryo confinement. Tag specificity was demonstrated by the simultaneous observation of 2-photon fluorescence emission and second harmonic generation (SHG) from myosin. Single molecule detection used highly inclined and laminated optical sheet (HILO) illumination and was verified by quantized photoactivation or photobleaching. Single molecule emission patterns from relaxed muscle indicated a highly orientationally confined lever-arm orientation. Results demonstrate detection of single myosin orientation *in vivo*. The zebrafish muscle system serves as an *in vivo* model for human disease and aging effects on myosin. Research supported by NIH R01AR049277 and R01HL095572.

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Protein Recognition and Selection through Conformational and Mutually Induced Fit

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Protein-protein interactions drive most every biological process but in many instances the domains mediating recognition are disordered. How specificity in binding is attained in the absence of defined structure contrasts with well-established experimental and theoretical work describing ligand binding to protein. The signaling protein calmodulin presents a unique opportunity to investigate mechanisms for target recognition given that it interacts with several